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(21) International Application Number: PCT/US98/25265 (22) International Filing Date: 25 November 1998 (25.11.98) (30) Priority Data: 979,456 26 November 1997 (26.11.97) US (71) Applicant: KERAPLAST TECHNOLOGIES, LTD. [US/US]; Suite 404, 2391 Northeast Loop 410, San Antonio, TX 78217 (US). (72) Inventors: BLANCHARD, Cheryl, R.; 2110 Flamingo Drive, San Antonio, TX 78209 (US). TIMMONS, Scott, F.; 9119 Bridlewood Lane #1, San Antonio, TX 78240 (US). SMITH, Robert, A.; 2246 E. Northside Drive, Jackson, MS 39211 (US). (74) Agents: SEAGER, Glenn, M. et al.; Crompton, Seager & Tufte, LLC, Suite 895, 331 Second Avenue South, Minneapolis, MN 55401 (US).		(81) Designated States: CA, JP, MX, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: KERATIN-BASED HYDROGEL FOR BIOMEDICAL APPLICATIONS AND METHOD OF PRODUCTION (57) Abstract A keratin hydrogel which can be used as a wound dressing and cell scaffolding. The keratin hydrogel is formed from clean, washed hair by partially oxidizing a significant percentage of disulfide linkages to form cysteic acid groups, while some disulfide linkages remain intact. The partially oxidized hair is treated with a reducing agent, thereby reducing most of the remaining disulfide linkages to cysteine-thioglycollate disulfide and cysteine groups. A soluble fraction of hair is collected and oxidized, such that the reduced sulfur groups are allowed to reform disulfide linkages, thereby binding the keratin together. The cysteic acid groups remain, providing hydrophilic sites within the hydrogel. A higher degree of partial oxidation results in a greater abundance of hydrophilic cysteic acid groups in the hydrogel.		

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KERATIN-BASED HYDROGEL FOR BIOMEDICAL APPLICATIONS AND METHOD OF PRODUCTION

Cross-Reference to Related Applications

The present application is related to U.S. Patent Application Serial No. _____, filed on date even herewith, entitled KERATIN-BASED SHEET MATERIAL FOR BIOMEDICAL APPLICATIONS AND METHOD OF PRODUCTION. The present application is also related to U.S. Patent No. 5,358,935, entitled NONANTIGENIC KERATINOUS PROTEIN MATERIAL, both herein incorporated by reference.

Field of the Invention

The present invention is related to wound dressing materials and tissue engineering scaffolds. More specifically, the present invention is related to a cross-linked keratin hydrogel.

Background of the Invention

Chronic wounds can be caused by a variety of events, including surgery, prolonged bedrest and traumatic injuries. Partial thickness wounds can include second degree burns, abrasions, and skin graft donor sites. Healing of these wounds can be problematic, especially in cases of diabetes mellitus or chronic immune disorders. Full thickness wounds have no skin remaining, and can be the result of trauma, diabetes (e.g., leg ulcers) and venous stasis disease, which can cause full thickness ulcers of the lower extremities. Full thickness wounds tend to heal very

slowly. Proper wound care technique including the use of wound dressings is extremely important to successful chronic wound management. Chronic wounds affect an estimated four million people a year, resulting in health care costs in the billions of dollars. "Treatment of Skin Ulcers with Cultivated Epidermal Allografts," T. Phillips, O. Kehinde, and H. Green, J. Am. Acad. Dermatol., V. 21, pp. 191-199 (1989).

The wound healing process involves a complex series of biological interactions at the cellular level which can be grouped into three phases: hemostasis and inflammation; granulation tissue formation and reepithelization; and remodeling. "Cutaneous Tissue Repair: Basic Biological Considerations," R.A.F. Clark, J. Am. Acad. Dermatol., Vol. 13, pp. 701-725 (1985). Keratinocytes (epidermal cells that manufacture and contain keratin) migrate from wound edges to cover the wound. Growth factors such as transforming growth factor- β (TGF- β) play a critical role in stimulating the migration process. The migration occurs optimally under the cover of a moist layer. Keratins have been found to be necessary for reepithelization. Specifically, keratin types K5 and K14 have been found in the lower, generating, epidermal cells, and types K1 and K10 have been found in the upper, differentiated cells. Wound Healing: Biochemical and Clinical Aspects, I.K. Cohen, R.F. Diegleman, and W.J. Lindblad, eds., W.W. Saunders Company, 1992. Keratin types K6 and K10 are believed to be present in healing wounds, but not in normal skin. Keratins are major structural proteins of all epithelial cell types and appear to play a major role in wound healing.

An optimum wound dressing would protect the injured tissue, maintain a moist environment, be water permeable, maintain microbial control, deliver healing agents to the wound site, be easy to apply, not require frequent changes and be non-toxic and non-antigenic. Although not ideal for chronic wounds, several wound dressings are currently on the market, including occlusive dressings, non-adherent dressings, absorbent dressings, and dressings in the form of sheets, foams, powders and gels. Wound Management and Dressing, S.Thomas, The Pharmaceutical Press, London, 1990.

Attempts have been made to provide improved dressings that would assist in the wound healing process using biological materials such as growth factors. To date, these biologicals have proven very costly and shown minimal clinical relevance in accelerating the chronic wound healing process. In cases of severe full thickness wounds, autografts (skin grafts from the patient's body) are often used. Although the graft is non-antigenic, it must be harvested from a donor site on the patient's body, creating an additional wound. In addition, availability of autologous tissue may not be adequate. Allografts (skin grafts from donors other than the patient) are also used when donor sites are not an option. Allografts essentially provide a "wound dressing" that provides a moist, water permeable layer, but is rejected by the patient usually within two weeks and does not become part of the new epidermis.

What would be desirable and has not heretofore been provided is a wound dressing that protects the injured tissue, maintains a moist environment, is water permeable, is easy to apply, does not require frequent changes and is non-toxic and

non-antigenic, and most important, delivers effective healing agents to the wound site.

Tissue engineering is a rapidly growing field encompassing a number of technologies aimed at replacing or restoring tissue and organ function. The consistent success of a tissue engineered implant rests on the invention of a biocompatible, mitogenic material that can successfully support cell growth and differentiation and integrate into existing tissue. Such a scaffolding material could greatly advance the state of the tissue engineering technologies and result in a wide array of tissue engineered implants containing cellular components such as osteoblasts, chondrocytes, keratinocytes, and hepatocytes to restore or replace bone, cartilage, skin, and liver tissue respectively.

Summary of the Invention

The present invention includes a hydrogel formed of cross-linked keratin not requiring an added binding agent. The hydrogel is believed to be bound together by reformed disulfide linkages and hydrogen bonds. A preferred use of the hydrogel is as a wound healing agent. Another preferred use is as a tissue engineering cell scaffold for implant applications. Yet another preferred use is as a skin care product.

The hydrogel can be formed from a soluble protein fraction derived from hair. Keratin can be obtained from a number of sources including human or animal hair, and finger or toe nails, with one source being hair of the patient or donors.

The hydrogel can be formed by providing clean, washed, rinsed, and dried hair. The hair is partially oxidized with an oxidizing agent such as peracetic acid.

The partial oxidation cleaves some disulfide linkages while leaving others intact.

The cleaved bonds can form sulfonic acid residues. The partially oxidized hair can be recovered with filtration, rinsed with deionized water, dried under vacuum, and ground to a powder.

The partially oxidized powder can then have some of the remaining intact disulfide linkages cleaved with a reducing agent such as ammonium thioglycollate in ammonium hydroxide by suspending the powder in such a reducing solution. The protein suspension can be heated to about 60° for about 4 hours and cooled to room temperature. The cleaved disulfide linkages are reduced to form cysteine groups and cysteine-thioglycollate disulfide groups, solubilizing the protein even further. The insoluble keratin fraction is preferably removed from the suspension by centrifuging the suspension and collecting the supernatant. The supernatant is preferably purified using a method such as dialysis. The supernatant can be further concentrated, in one method, by application of vacuum at ambient or sub-ambient temperatures.

The supernatant, having keratin with sulfonic acid groups, cysteine groups, and cysteine-thioglycollate disulfide groups, is now oxidized to allow formation of disulfide linkages between protein backbones. The sulfonic acid residues remain as hydrophilic sites within the protein. The hydrophilic sites bind water in the hydrogel. The hydrogel is thus formed of pure keratin, bound together with disulfide linkages and hydrogen bonds. The hydrogel requires no binders. The keratin hydrogel provides a non-antigenic, mitogenic wound healing agent that maintains wound moisture and provides a scaffold for cell growth for tissue engineered implants. Another application for this keratin gel is as a skin care product.

Keratin has been shown to be biocompatible, non-immunogenic, not to inhibit activated T-cells and therefore not interfere with the normal cell mediated immune response, and to be mitogenic for keratinocytes, fibroblasts, and human microvascular endothelial cells. Keratin has also been shown to promote epithelialization in wound healing studies on rats and humans.

Detailed Description of the Preferred Embodiments

In a method according to the present invention, hair is provided, preferably washed and unbleached. The hair is harvested from a human or animal source. The patient or a human donor is a preferred source of hair, as hair from these sources is most likely to result in a non-antigenic wound healing product, although animal hair may be acceptable for certain individuals that do not have animal product allergy problems. In one method, the hair is washed with Versa-Clean TM (Fisher Scientific, Pittsburgh, PA), rinsed with deionized water, and allowed to air dry.

The hair can be oxidized in peracetic acid or another suitable reagent such as H_2O_2 . A preferable treatment utilizes from 1% to 32% peracetic acid, at a temperature between about 0°C and 100°C for between 0.5 and 24 hours. One method treats 30 grams of hair with 500 mL of 32% peracetic acid at 4°C for 24 hours. This treatment with peracetic acid is believed to partially oxidize the naturally occurring disulfide linkages to produce a protein with cysteic acid ($-CH_2SO_3H$) residues, and remaining disulfide linkages.

The hair is recovered, preferably with filtration through a coarse fritted glass filter, and rinsed numerous times with deionized water until the rinse solution has a

pH of 6.0 or higher. The hair can then be dried in a vacuum oven at between 20°C and 50°C for between 0.5 and 5 days. One method dries the hair in a vacuum oven at 40°C for several days. The dried hair can then be pulverized and ground into a fine powder. One method of grinding the hair uses a ceramic mortar and pestle.

The keratin powder can be suspended in ammonium thioglycollate. In one method, pulverized keratin powder, derived from hair as described above, is suspended in about 3N ammonium hydroxide containing ammonium thioglycollate. About six grams of keratin powder can be added per 75 mL of ammonium hydroxide. The strength of ammonium hydroxide is preferably about 3N and the preferred concentration of ammonium thioglycollate is about 11 mL (as thioglycollic acid) per 75 mL of ammonium hydroxide. The suspension can then be heated for a time sufficient to solubilize the soluble fraction of the hair. The suspension in one method is heated between 50° and 90°C for between 1 and 24 hours, followed by cooling. In a preferred method, the suspension is heated to about 60°C for about 4 hours and cooled to room temperature. Applicants believe this treatment cleaves the remaining disulfide linkages to produce cysteine residues in the protein structure. At this point, the keratin protein is believed to contain cysteic acid, cysteine and cysteine-thioglycollate disulfide residues. The ratio of cysteic acid residues and cysteine residues can be controlled by varying the time, temperature, and concentration of oxidant in the peracetic acid treatment step previously described. The presence of sulfonic acid residues imparts a hydrophilic property to the hair as well as to the final hydrogel product.

After the treatment described above, a keratin fraction resistant to the treatment remains, consisting primarily of Beta keratin. This fraction is insoluble in the suspension and is removed in one method by centrifugation at about 10,000 g for about 10 minutes. The insoluble fraction is set aside for other use. A thick, jelly-like supernatant remains which includes a soluble keratin fraction. The supernatant is collected.

The supernatant is preferably purified, using a method such as dialysis. A preferred method uses dialysis against running water using a dialysis membrane (Spectra/Por TM) having a cutoff of about 8000 MW. The resulting solution is preferably concentrated to a concentration of about 0.1 grams per mL.

The keratin in solution is now ready for cross-linking to form a hydrogel. In a preferred method, an oxidizing agent is added to the keratin to crosslink the keratin proteins. Preferred oxidizing agents include hydrogen peroxide, organic peracids, peroxy carbonates, ammonium sulfate peroxide, benzoyl peroxide, and perborates. Hydrogen peroxide is preferably added to the keratin solution at about 0.5% to about 1.0% w/v, mixed well, and allowed to stand at room temperature for several days. A preferred standing time is about 3 days. The freely flowing solution slowly thickens and converts to a cross-linked hydrogel after about 72 hours.

The soluble keratin fraction from hair is thus partially oxidized so as to have the protein backbones interconnected with disulfide linkages and having sulfonic acid residues. The partially oxidized keratin is treated with a reducing agent to cleave some or all of the remaining disulfide bonds, forming thiol groups and cysteine-thioglycollate disulfide groups and solubilizing more of the keratin proteins. After

removing the insoluble fraction, the keratin is oxidized to allow the formation of disulfide cross-links. Disulfide cross-links are thus reformed. As used herein, the term "reformed" refers to cross-links broken and formed later in time, where individual linkages later formed could be, but are not necessarily, between the same amino acid cysteine pairs.

A cross-linked, pure keratin hydrogel results. The hydrogel has sulfonic acid groups which are hydrophilic and bind water within the hydrogel. The number of sulfonic acid groups corresponds to the degree of keratin oxidation in the partial oxidation step.

Applicants believe the keratin product made according to this method is suitable for use as a wound healing agent and as a mitogenic cell growth scaffold for tissue engineering applications and as a nutrient support for cell growth. It is also suitable for skin care applications. Anti-bacterial additives, ointments and biologicals such as growth factors or collagen can be added to the keratin hydrogel.

Experimental Results

A keratin-based hydrogel wound healing agent not requiring binder material was prepared from keratin derived from human hair. Human hair was obtained from males aged 12 to 20 years, washed with Versa-Clean TM (Fisher Scientific, Pittsburgh, PA), rinsed with deionized water and allowed to air dry. This hair was subsequently chopped into approximately 0.25 inch to 2 inch lengths using shears. Thirty grams of this hair was treated with 500 mL of 32% peracetic acid (Aldrich Chemical, Milwaukee, WI) at 4°C for 24 hours. This treatment partially oxidized the

disulfide linkages. The hair was recovered by filtration through a coarse fritted glass filter and rinsed numerous times with deionized water until the rinse solution was pH 6.0 or higher. The hair was dried under vacuum at 40°C for several days until completely dry and ground to a fine powder with a ceramic mortar and pestle. The resulting material, 19 grams, was used to produce a keratin hydrogel.

Six grams of the pulverized, oxidized hair was suspended in 75 mL of 3N ammonium hydroxide containing 11 mL of ammonium thioglycollate (as thioglycollic acid). The suspension was heated to 60°C for 4 hours and then cooled to room temperature. This treatment cleaved any remaining disulfide linkages to produce cysteine and cysteine-thioglycollate disulfide residues in the protein structure. An insoluble fraction remained which was resistant to solubilization by the ammonium hydroxide and ammonium thioglycollate. The insoluble fraction, believed to be comprised mostly of Beta-keratin, was isolated by centrifugation at 10,000 g for 10 minutes. A thick, jelly-like supernatant was removed from the centrifuged material, with the insoluble material set aside for use in a related product.

The supernatant was dialyzed for 72 hours against running water using a dialysis membrane with an 8000 MW cutoff (Spectra/Por™). The resulting solution was concentrated to 50 mL, in-vacuo at sub-ambient temperature. The solution was treated with 3% hydrogen peroxide added at a rate of 0.5% to 1.0% w/v mixed well and allowed to stand at room temperature for 3 days. The freely flowing solution slowly thickened and converted to a crosslinked hydrogel after 72 hours. The hydrogel can be used as a wound healing agent or a cell scaffold.

The use of keratin-containing materials in promoting wound healing was demonstrated in several experiments. In a first experiment, processed human hair was incubated with cell culture media. The media/hair mixture was passed through a micro filter. Cell lines relevant to wound healing, including human microvascular endothelial cells, keratinocytes and fibroblasts, were placed in culture using this media extract. Significant proliferation of these wound healing cells was measured. Keratinocytes proliferated profusely, fibroblasts proliferated modestly, and endothelial cells proliferated profusely.

The mitogenic activity observed in fibroblast, keratinocyte, and endothelial cell cultures is additional evidence that the keratinous protein material is not only biocompatible but also mitogenic with these cell lines. Additional biocompatibility was observed when keratin microfibrils were observed microscopically to be in direct contact with cells in the cell cultures. Specifically, keratinocytes and fibroblasts were observed to adhere to and congregate around microfibrils indicating that desirous cell activity can be sustained on this naturally derived biopolymer matrix.

In a second experiment, processed human hair powder was incubated with cell culture media. The media/keratin mixture was passed through a micro filter. This media extract was used in proliferation studies with lymphocytes. The lymphocyte cell line did not proliferate, indicating the material to be non-immunogenic.

In a third experiment, processed human hair powder was incubated with cell culture media. The media/hair mixture was then passed through a micro filter. This media extract was used in proliferation studies with activated T-lymphocytes. The

T-lymphocytes proliferated normally, indicating no inhibition of the normal cell-mediated immune response by the keratin. This demonstrated no inhibition of this very important function of immune cells.

In a fourth experiment, human hair was chemically treated. This produced a keratin slurry that was then cast into a sheet and chemically crosslinked to produce a non-soluble sheet of keratin. Segments of the sheeting were then incubated with keratinocytes, fibroblasts and human microvascular endothelial cells. These cells were shown to grow and proliferate favorably on the keratin sheet. This indicates that skin component cells proliferate favorably in the presence of keratin sheeting produced by the above described method.

In a fifth experiment, twenty-eight hairless rats were wounded on either side of the dorsal midline with a dermatome, creating a partial thickness wound, 0.12 inches in depth, and 2.0 x 4.0 cm in surface area. Half the wounds were treated with keratin powder, half were not, and both halves were covered with polyurethane dressing. The wounds were observed for healing and biopsied at days 0, 2, 4 and 6 for histochemical analysis. Planimetry studies showed 97% epithelialization of the keratin treated wounds and 78% epithelialization of the non-treated wounds at day 4. Histological analysis by H & E stain revealed total epithelialization microscopically of the keratin treated wounds at day 2 and only partial epithelialization of the non-treated wounds at day 2. Histological analyses at days 4 and 6 also revealed an acceleration of the epithelialization maturation process in the keratin treated wounds.

Human clinical studies are currently being performed on donor sites for skin grafts. One half of the donor wound site is treated with sterilized keratin powder and the opposite half treated in a standard fashion, with Adaptic™ non-adhering dressing from Johnson & Johnson. Preliminary results show the keratin treated halves epithelialize sooner and mature more rapidly. This was confirmed through both clinical observations and histological results of 4 millimeter punch biopsies. Subjectively, patients also have much less pain in the keratin treated wounds.

Numerous characteristics and advantages of the invention covered by this document have been set forth in the foregoing description. It will be understood, however, that this disclosure is, in many respects, only illustrative. Changes may be made in details, particularly in matters of shape, size, and ordering of steps without exceeding the scope of the invention. The invention's scope is, of course, defined in the language in which the appended claims are expressed.

What is claimed is:

1. A keratin hydrogel resulting from the process comprising the steps of:
providing hair, wherein said hair is formed of keratin having disulfide linkages;

partially oxidizing said hair with a first oxidizing agent, such that a significant percent of said disulfide linkages in said keratin are converted to cysteic acid groups and a significant percent of said disulfide linkages remain intact;

reducing said hair with a reducing agent, such that a significant percentage of said remaining disulfide linkages are reduced to cysteine groups and cysteine-thioglycollate disulfide groups; and

oxidizing said hair with a second oxidizing agent, such that a significant percentage of said reduced cysteine and cysteine-thioglycollate disulfide groups are oxidized to reform disulfide linkages, wherein the reformed disulfide linkages are not necessarily the same disulfide linkages existing in the hair provided;

such that said hydrogel is bound together by said reformed disulfide linkages and has cysteic acid groups, wherein said cysteic acid groups are hydrophilic.

2. A keratin hydrogel as recited in claim 1 wherein between about 1 % and 90 % of said disulfide linkages are oxidized in said partial oxidation step.

3. A keratin hydrogel as recited in claim 1 wherein between about 50 % and 100 % of said remaining disulfide linkages are reduced in said reduction step.

4. A keratin hydrogel as recited in claim 1 wherein between about 1 % and 100 % of said reduced groups are oxidized to reform disulfide linkages in said oxidation step.

5. A keratin hydrogel as recited in claim 1 wherein between about 1 % and 90 % of said disulfide linkages are oxidized in said partial oxidation step;

between about 50 % and 100 % of said remaining disulfide linkages are reduced in said reduction step; and

between about 1 % and 100 % of said reduced groups are oxidized to reform disulfide linkages in said oxidation step.

6. A keratin hydrogel as recited in claim 1 wherein said first oxidizing agent includes peracetic acid and said hair is reduced with an alkaline thioglycollate solution.

7. A keratin hydrogel as recited in claim 6 wherein said second oxidizing agent is selected from the group consisting of hydrogen peroxide, organic peracids, peroxy carbonates, ammonium sulfate peroxide, benzoyl peroxide, and perborates.

8. A keratin hydrogel resulting from the process comprising the steps of: providing clean, washed hair;

oxidizing said hair with about 32% peracetic acid at about 4°C for up to about 24 hours;

recovering said hair by filtering;

rinsing said hair with deionized rinse water until said rinse water has a pH of at least about 6.0;

drying said hair under ambient conditions or vacuum at about 40°C until completely dry;

grinding said hair to a fine powder;

suspending said powder in a solution of about 3N ammonium hydroxide containing about 11 mL of ammonium thioglycollate as thioglycollic acid per 75 mL of ammonium hydroxide, thereby forming a suspension;

heating said suspension to about 60° C for about 4 hours;

cooling said suspension to room temperature;

centrifuging said suspension at about 10000 g for about 10 minutes and collecting the supernatant;

purifying said supernatant with dialysis against running water for about 72 hours using a dialysis membrane having about a 8000 MW cutoff;

concentrating said purified supernatant;

oxidizing said concentrate by adding about 3% hydrogen peroxide at a rate of about 0.5% to about 1.0% w/v and mixing well; and

allowing said concentrate to stand at room temperature for about 72 hours, thereby forming a crosslinked hydrogel.

9. A process for forming a keratin hydrogel comprising the steps of:
providing hair, wherein said hair is formed of keratin having initial disulfide linkages;
reacting said hair with a first agent that adds hydrophilic groups to said keratin;
reducing said hair with a reducing agent, such that a significant percentage of said disulfide linkages are converted to reduced groups; and
oxidizing said hair with a second agent, such that a significant percentage of said reduced groups are oxidized to reform disulfide linkages,
such that said hydrogel is bound together by said reformed disulfide linkages and has hydrophilic groups.

10. A process for forming a keratin hydrogel as recited in claim 9 wherein said first agent is a first oxidizing agent and said hair is partially oxidized with said first oxidizing agent, such that a significant percent of said disulfide linkages in said keratin are oxidized to form said hydrophilic groups and a significant percent of said disulfide linkages remain un-oxidized.

11. A process for forming a keratin hydrogel as recited in claim 10 wherein
a significant percent of said disulfide linkages in said keratin are oxidized to cysteic acid groups in said partial oxidation step, and

said reduced groups include cysteine groups and cysteine-thioglycollate disulfide groups,

such that said hydrogel is bound together by said reformed disulfide linkages and has cysteic acid groups, wherein said cysteic acid groups are hydrophilic.

12. A process for forming a keratin hydrogel comprising the steps of:
providing vertebrate hair;
oxidizing said hair;
suspending said oxidized hair in a solution of ammonium hydroxide containing ammonium thioglycollate, thereby forming a suspension;
separating a soluble fraction of keratin from said suspension;
adding an oxidizing agent to said soluble keratin fraction; and
allowing said concentrate to stand, thereby forming a crosslinked hydrogel.

13. A process for forming a keratin hydrogel as recited in claim 12 wherein said separation step includes centrifuging said suspension and collecting a supernatant.

14. A process for forming a keratin hydrogel as recited in claim 13 further comprising purifying said supernatant by dialysis.

15. A process for forming a keratin hydrogel as recited in claim 14 further comprising heating said suspension prior to centrifuging.

16. A process for forming a keratin hydrogel as recited in claim 15 wherein said suspension is heated to a temperature of about 60°C.

17. A process for forming a keratin hydrogel as recited in claim 12 further comprising concentrating said soluble fraction.

18. A process for forming a keratin hydrogel as recited in claim 17 wherein said concentrated fraction has a concentration of about 0.1 grams of keratin per mL.

19. A wound healing agent comprising a hydrogel formed of keratin protein, wherein said keratin protein is bound together with covalent, disulfide links, and said keratin protein has anionic groups responsible for the hydrophilic property.

20. A hydrogel formed from the steps comprising:
providing a concentrated keratin solution wherein said keratin has chemically produced hydrophilic groups, and cysteine groups;
adding an oxidizing agent to said keratin solution; and
allowing said keratin solution to crosslink, such that at least some of said cysteine groups establish disulfide bonds therebetween, thereby forming a crosslinked hydrogel having said hydrophilic groups.

21. A hydrogel as recited in claim 20 wherein said chemically produced hydrophilic groups include cysteic acid groups.
22. A hydrogel as recited in claim 21 wherein said keratin includes cysteine-thioglycollate disulfide groups.
23. A hydrogel as recited in claim 20 wherein said concentrated hydrogel has a concentration in the range of 0.05 to 0.4 grams of keratin per mL.
24. A hydrogel as recited in claim 22 wherein said oxidizing agent is selected from the group consisting of hydrogen peroxide, organic peracids, peroxy carbonates, ammonium sulfate peroxide, benzoyl peroxide, and perborates.
25. A hydrogel as recited in claim 24 wherein said oxidizing agent is hydrogen peroxide.
26. A hydrogel as recited in claim 20 wherein said keratin is an anionic polyelectrolyte keratin at physiological pH or higher.
27. A hydrogel as recited in claim 21 wherein said gel does not include a binder.
28. A method for treating a wound comprising the steps:

providing a keratin hydrogel wherein said hydrogel is bound together with covalent, keratin disulfide linkages; and
applying said hydrogel to said wound.

29. A method for treating a wound as recited in claim 28 wherein said keratin hydrogel is bound together primarily with disulfide bonds and includes hydrophilic cysteic acid residues.

30. A keratin hydrogel resulting from the process comprising the steps of:
providing keratin, wherein said keratin has initial disulfide linkages;
chemically treating said keratin with a first reagent, such that hydrophilic groups are produced in said keratin;
reducing said keratin with a reducing agent, such that a significant percentage of said disulfide linkages are converted to reduced groups; and
oxidizing said keratin with an oxidizing agent, such that a significant percentage of said reduced groups are oxidized to reform disulfide linkages,
such that said hydrogel is bound together by said reformed disulfide linkages and has hydrophilic groups.

31. A keratin hydrogel as recited in claim 30 wherein said first reagent is an oxidizing agent, said hydrophilic groups include cysteic acid formed from said initial disulfide linkages, and some of said initial disulfide linkages remain intact after said chemical treatment step.

32. A keratin hydrogel as recited in claim 31 wherein said reduced groups include cysteine.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/25265

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : 424/70.1; 514/21; 525/54.1; 530/357

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/70.1; 514/21; 525/54.1; 530/357

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN

search terms: keratin hydrogel, keratin product, keratin and wound healing

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P --- A	US 5,763,583 A (ARAI et al) 09 June 1998, col. 1, line 35; col. 2, lines 16-20, 57-62; col. 3, lines 1-8, 24-28; col. 4, lines 1-3, 49-53.	1-7, 9-32 --- 8
Y	GB 0531446 A (BAKONYI) 03 January 1941, p.1, lines 28-73.	1-7, 9-32
Y	US 5,047,249 A (ROTHMAN et al) 10 September 1991, col. 3, lines 13-18, 30-42; col. 6, lines 36-44; col. 7, lines 27-57.	19, 28, 29

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* "A"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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